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Increased Binding of Concanavalin A to Interferon-Treated Murine Leukemia L 1210 Cells (38279)

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Interferon preparations exert various biologic effects on cells which can be measured in different ways: inhibition of viral replication (1); inhibition of replication of other intracellular parasites (2–5); inhibition of cell division (6–9); decreased tumorigenicity and decreased colony-forming capacity of L 1210 cells in agarose (10); enhanced phagocytosis by macrophages (11); enhanced cytotoxicity by sensitized lymphocytes (12); enhanced production of specific proteins, such as interferon (13, 14) or aryl hydrocarbon hydroxylase (15); and enhanced cellular sensitivity to the toxic effects of vaccinia virus (16, 17) and double-stranded polynucleotides (18).

The nature of some of these effects suggested the possibility that interferon treatment might be associated with modifications of the cell surface and that such modifications might play an important part in the expression of these effects. We have recently shown that interferon treatment of murine leukemia L 1210 cells is in fact accompanied by a marked enhancement of the expression of H-2 histocompatibility surface antigens (19). In pursuing our investigations on the effect of interferon on the cell surface, it was considered of interest to compare the binding of Concanavalin A (Con A) to untreated and interferon-treated L 1210 cells using radioactive-labeled Con A and peroxidase-labeled Con A for electron microscopic examination. The results of these experiments are presented herein.

Materials and Methods. The origin of lymphoid leukemia L 1210 cells (20) and the selection of the interferon-resistant subline L 1210-R (9) have been previously described, as well as the techniques of culture (nonagitated suspension culture) in nutrient medium RPMI 1640 with 20% heat-inactivated horse serum (7, 9). In most experiments, mouse interferon (specific activity of $\geq 10^6$ U/mg protein) was prepared from the nutrient medium of cultures of C-243 cells (21) inoculated with Newcastle Disease virus. The techniques of preparation and assay of this interferon, mouse L cell culture interferon, C57Bl/6 mouse brain interferon, rabbit RK 13 cell interferon, and control preparations have been previously described (8, 22) as well as the procedures for inactivation of interferon with trypsin, sodium periodate, and heat. One mouse interferon unit as expressed in the text equals 4 mouse interferon reference units.

In the experiments to be described, treated or untreated L 1210 cells were centrifuged, washed once in nutrient medium without serum, recentrifuged, and resuspended in phosphate-buffered saline (PBS) at a concentration of 2×10^6 cells/ml. The viability of L 1210 cells was determined by trypan blue dye exclusion in each experiment. In no instance did interferon significantly affect cell viability.

Concanavalin A was prepared by Miles Yeda (Israel) from Jack Bean Meal, twice crystallized and lyophilized. Radioactive nickel Con A (^{63}Ni–Con A, sp. act 5–10 $\times$ 10^6 cpm/mg) was prepared according to Kalb and Levitzki (23) and purified on a Sephadex G 75 column. ^{63}Nickel (3.6 Ci/g) was obtained from I.R.E. (Belgium). ^{63}Ni–Con A displayed the same migration rate as native Con A after gel electrophoresis and affinity chromatography on a G 75 Sephadex column. Cells were incubated for 30 min at 22° with 50 μg ^{63}Ni–Con A, centrifuged, washed 3 times with PBS, and the total radioactivity of the cell pellet after solubilization in NaOH was determined in an Intertechnique scintillation counter. ^{63}Ni–Con A showed the

same binding affinity as native Con A. Thus, competitive binding experiments done by mixing radioactive and unlabeled Con A showed that the procedures used for labeling did not induce any change in binding affinities (Table I) (*i.e.*, a 1:1 or a 1:9 mixture of "hot and cold" lectin produced 1/2 or 1/10th as much surface labeling as obtained with ^{63}Ni–Con A alone). Specificity of binding of ^{63}Ni–Con A for untreated (or interferon-treated L 1210 cells) was shown by an inhibition of over 95% of binding by addition of 0.1 *M* methyl-α-D-mannopyranoside (αMM) (24) (Table I). As a further control, similar competitive binding experiments were undertaken with ^{3}H-acetylated Con A (2×10^6 cpm/mg) (25) which yielded results identical to those obtained using ^{63}Ni–Con A.

For electron microscopic examination, viable control or interferon-treated L 1210 cells were incubated with Con A and then with peroxidase according to the technique of Bernhard and Avrameas (26). Glutaraldehyde fixation was performed in a 2.5% solution in PBS for 15 min at 22°. Peroxidase was detected after a 30-min incubation at 22° with 3.3′ diaminobenzidine (0.5 mg/ml) in a 0.1 *M* Tris buffer, pH 7.4, containing 0.01% H_2O_2 (27). Cells were then fixed in 2% osmium tetroxide for 1 hr, dehydrated in alcohol, and embedded in Epon. Sections were counter stained for 2 min with lead citrate and examined with a Philips EM 200 (objective aperture 40 μm, acceleration voltage 80 kV). To compare the different rates of pinocytosis between untreated and interferon-treated cells, Con A–peroxidase-labeled cells were reincubated at 37° before glutaraldehyde fixation (28).

TABLE I. Binding of ^{63}Ni–Con A to L 1210 Cells[a].

Lectins (50 μg/ml)	cpm/4 × 10^6 Cells
^{63}Ni–Con A alone	29,100
^{63}Ni–Con A + native Con A (Ratio of 1:1)	14,350
^{63}Ni–Con A + native Con A (Ratio of 1:9)	2,950
^{63}Ni–Con A + 0.1 *M* αMM	1,327

[a] L 1210 cells (2×10^6 cells/ml) were incubated with native or labeled ^{63}Ni–Con A for 30 min at 22°, washed × 3 in PBS, and the cell pellet was solubilized in NaOH (see Materials and Methods).

Results and Discussion. Preliminary experiments showed that cultivation of L 1210 cells for 24 hr with interferon (1600 U/ml) resulted in a slight increase in ^{63}Ni–Con A binding compared to control cultures, whereas a much greater increase in binding was observed after 48 hr of cultivation with interferon. Accordingly, in the experiments to be described, we have determined the Con A binding to cells cultivated for 48 hr with 1600 U of interferon/ml (a significant effect of interferon can also be observed with lesser amounts—*i.e.*, even 160 U). Figure 1A illustrates the inhibitory effect of interferon on the multiplication of L 1210 cells and the increased binding of Con A (per 2×10^6 cells) to interferon-treated cells compared with its binding to untreated L 1210 cells. (Increased binding of Con A to interferon-treated L 1210 cells was also observed when expressed as μg Con A/mg cell protein instead of per 2×10^6 cells.) In a series of nine experiments, the range of binding of ^{63}Ni–Con A to untreated L 1210 cells at 48 hr of cultivation was 1.72–3.38 μg (mean 2.53 μg) per 2×10^6 cells, whereas the range of binding of ^{63}Ni–Con A to interferon-treated cells was 4.2–7.1 μg (mean 4.97) per 2×10^6 cells. As can be seen in Fig. 1B, interferon treatment did not affect the binding of Con A to interferon-resistant L 1210 cells.

Treatment of L 1210 cells with different mouse interferon preparations derived from C-243 or L cells or mouse brain all proved effective, as did a highly purified mouse cell culture interferon (1×10^7 U/mg protein) provided through the courtesy of Dr. Bodo (29). The increased binding of Con A observed in interferon-treated cultures was not observed when the interferon preparation was pretreated with trypsin or sodium periodate which also destroyed its effect on cell division and on viral replication (Fig. 2). A control preparation and the heterologous rabbit interferon were ineffective (Fig. 2). Other experiments showed that a highly purified human interferon (2×10^6 U/mg protein received through the courtesy of Dr. Kishida) also proved ineffective.

To determine whether the increased binding of radioactive Con A to interferon-treated cells was due to an increased binding at the cell surface or an increased intracellular uptake of labeled Con A, control and interferon-treated cells were examined by electron microscopy (using peroxidase-labeled Con A) at the time

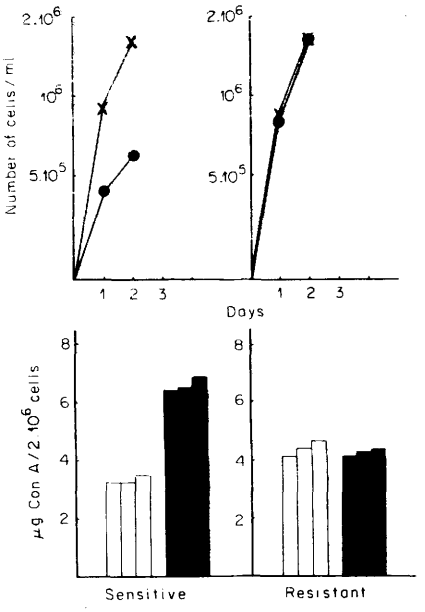

FIG. 1. Three cultures of interferon-sensitive and interferon-resistant L 1210 cells cultivated in the absence of interferon (X) or in the presence of 1600 U/ml of mouse C-243 cell interferon (●).

Binding of Con A on cells after 48 hr of cultivation: open bar (□) refers to untreated cells from one culture and closed bar (■) to interferon-treated cells from one culture.

radioactive Con A binding was determined. At this time, all the label was observed at the cell surface on both control (Fig. 3A) and interferon-treated cells (Fig. 3B), and no pinocytosis of peroxidase-labeled Con A was detected. (However, when unfixed Con A-peroxidase-labeled cells were postincubated at 37°, pinocytosis did occur in both control and interferon-treated cells. No difference in the rate of pinocytosis was observed between these cultures during a 2-hr incubation time.)

Cikes and his coworkers (30–33) found that the expression of surface antigens of a mouse leukemia cell line varied considerably during the growth cycle, but in general antigenic concentration increased as the growth rate declined. We have obtained comparable results in our previous studies with L 1210 cells (19). Although we conclude from the results presented herein that treatment of L 1210 cells with interferon is accompanied by an increased binding of Con A, our results do not permit us to state whether this effect is independent of or related to the inhibitory effect of interferon on cell division. In the experiments presented above, both control cultures and cells to be treated with interferon were seeded at 2×10^5 cells/ml. In other experiments, control cells and cells to be treated were seeded at different concentrations so that at the time of testing (*i.e.*, 24, 48, or 72 hr), all cultures would still be in exponential growth phase. Under these conditions, a clearcut increase in Con A binding per cell or per mg cell protein was again seen after 48 and 72 hr of cultivation of cells with interferon.

An association has been suggested between Con A binding and malignancy (34–38), although the validity of this hypothesis has been questioned (39, 40). We have previously reported that interferon treatment of L 1210 cells was associated with a decreased tumorigenicity for DBA/2 mice and a decreased colony-forming capacity in agarose (10). It was considered of

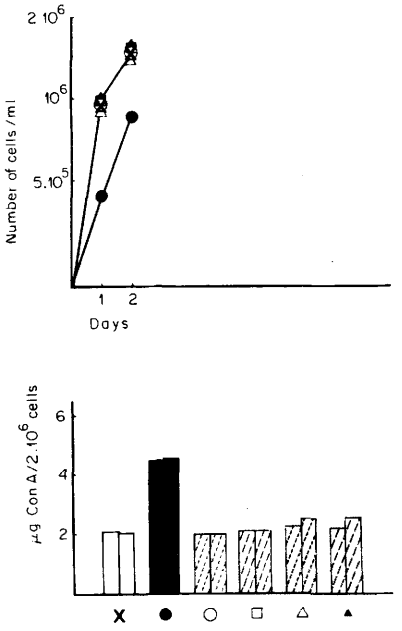

FIG. 2. Two cultures of interferon-sensitive L 1210 cells cultivated in the absence of interferon (X), in the presence of 1600 U/ml of mouse C-243 cell interferon (●), in the presence of mock C-243 interferon (○), or in the presence of interferon pretreated with trypsin (□) or sodium periodate (△), or in the presence of rabbit RK 13 cell culture interferon (▲)

The original antiviral activity of each preparation as assayed on mouse L cells challenged with VSV was as follows: mouse interferon 1:16,000, mock C-243 interferon < 1:10, Typsin-treated interferon < 1:10, Sodium-periodate-treated interferon < 1:10, rabbit interferon < 1:10 (titer on rabbit RK 13 cells 1:8,000).

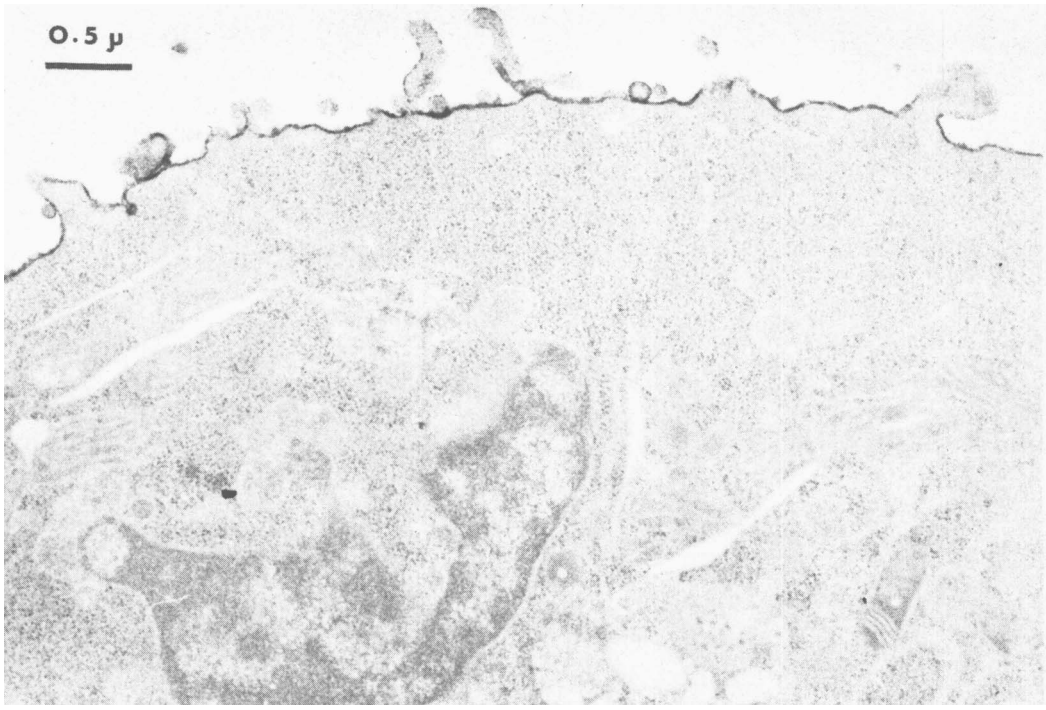

FIG. 3A: Control L 1210 cells labeled after 48 hr of culture with Con A–peroxidase (22.500 ×). 3B: Interferon-treated L 1210 cells labeled after 48 hr of culture with Con A–peroxidase. (22.500 ×); insert (280.000 ×)

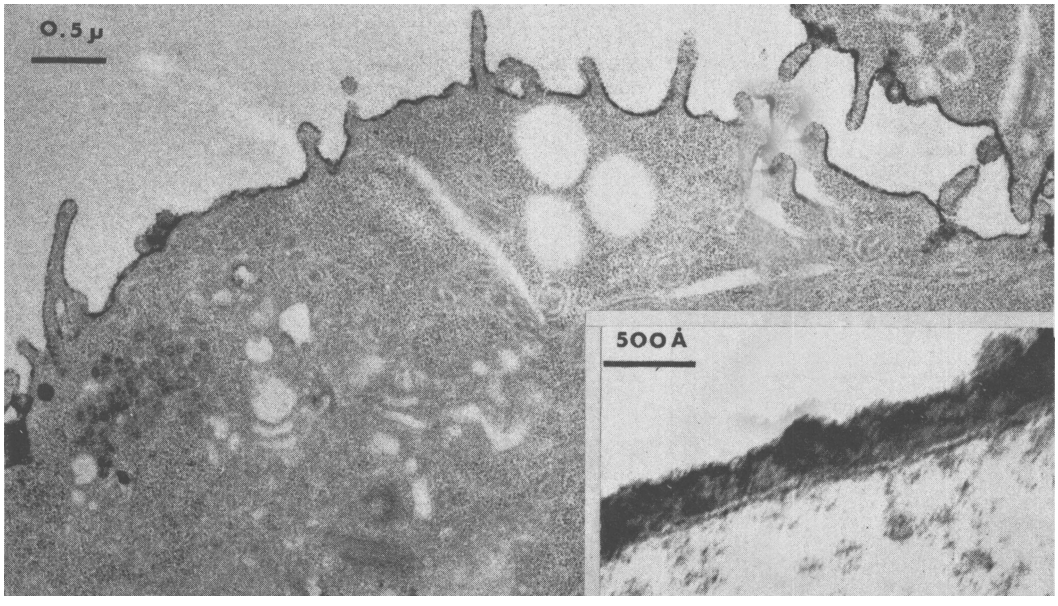

TABLE II. Effect of Interferon Treatment on the Binding of ^{63}Ni–Con A and the Tumorigenicity of L 1210 Cells.

		Control	Interferon treated
^{63}Ni–Con A binding[a]		1.72	4.9
μg/2 × 10^6 cells		1.97	5.4
Tumorigenicity[b]	10^5	7/7	10/10
for DBA/2 mice	10^4	10/10	6/10
(No. of cells inoculated ip/mouse)	10^3	10/10	2/10
	10^2	10/10	2/9
	10^1	10/10	0/10

[a] Two control cultures and 2 interferon treated cultures were examined at 48 hr for Con A binding.
[b] Numerator = number of mice dead with tumor at 60 days/total number of mice injected.

interest to determine in the same experiment the effect of interferon on Con A binding and tumorigenicity of L 1210 cells. As can be seen from Table II, interferon-treated (48 hr) L 1210 cells displayed more than a twofold greater capacity to bind Con A but at the same time exhibited a marked decrease in tumorigenicity compared to control L 1210 cells.

Our previous work indicated that cell-surface changes in interferon-treated L 1210 cells could be measured as an enhanced expression of histocompatibility antigens (19). The most likely explanation of the results presented herein is that modifications of the surface of interferon-treated L 1210 cells also result in an increased binding of Con A to the cell surface. Such cell surface changes would seem important in understanding the many effects of interferon on animal cells.

Summary. Cultivation of mouse lymphoid leukemia L 1210 cells with mouse interferon preparations resulted in an increased binding of radioactive Concanavalin A (Con A). No increase in Con A binding was observed when L 1210 cells were cultivated with control preparations, inactivated mouse interferon preparations, or heterologous interferon preparations, nor when a subline of interferon resistant L 1210 cells was cultivated with mouse interferon. Electron microscopic examination of control or interferon-treated L 1210 cells using peroxidase-labeled Con A showed that all the label was seen at the cell surface. Interferon-treated L 1210 cells exhibiting an increased binding of Con A were far less tumorigenic for DBA/2 mice than untreated L 1210 cells. We conclude that interferon treatment of L 1210 cells is accompanied by modifications of the cell surface which can be detected by an increased binding of Con A to the cell surface.

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